

Claims

1. An osteogenic device for implantation in a mammal, said device comprising:

a biocompatible, in vivo biodegradable matrix defining pores of a dimension sufficient to permit influx, proliferation and differentiation of migratory progenitor cells from the body of said mammal; and

a protein, produced by expression of recombinant DNA in a host cell, comprising one or more polypeptide chains, each of which has an amino acid sequence sufficiently duplicative of the sequence of COP-5 or COP-7 such that said protein is capable of inducing endochondral bone formation in association with said matrix when implanted in a mammal.

2. A device for implantation in a mammal, said device comprising:

a biocompatible, in vivo biodegradable matrix defining pores of a dimension sufficient to permit influx, proliferation and differentiation of migratory progenitor cells from the body of said mammal; and

a protein, produced by expression of recombinant DNA in a host cell, comprising one or more polypeptide chains, each of which has less than about 200 amino acids, in a sequence sufficiently duplicative of the sequence of

COP-5 or COP-7 such that said protein is capable of inducing cartilage formation in association with said matrix when implanted in a mammal.

3. The device of claim 1 or 2 wherein the sequence comprises:

10	20	30	40	50
CXXXXLXVXF	DXGWXXWXX	XPXGX	AXYCXGXCXX	PXXXXXXXNHAXX
60	70	80	90	100
QXXVXXNXX	XXPXCCXP	XXXXXXXXL	XXXXXXXXVXL	XXYXXMXVXXCXCX

wherein each X independently represents an amino acid.

4. The device of claim 1 or 2 wherein the sequence comprises:

10	20	30	40	50
LXVXF	DXGWXXWXX	XPXGX	AXYCXGXCXX	PXXXXXXXNHAXX
60	70	80	90	100
QXXVXXNXX	XXPXCCXP	XXXXXXXXL	XXXXXXXXVXL	XXYXXMXVXXCXCX

wherein each X independently represents an amino acid.

5. The device of claim 1 or 2 wherein the sequence comprises:

10	20	30	40	50
CKRHPLYVDF	RDVGW	NDWIVAP	PGYHAFYCH	GECPPFLADHLNSTNHAIV
RRRS K S S L	QE VIS E	FD Y E A	AY MPESMKAS	VI
KE F E K I	DN L N S	Q ITK F P	TL	
Q A S K				
60	70	80	90	100
QTLVNSVNP	GKIPKACCV	PTELSAIS	MLYLDENEN	VVLKNYQDMVVEGCGCR
SI HAI	SEQV EP A	EQMNSLAI	FFNDQDK I	RK EE T DA H H
RF T S	K DPV V	Y N S	H RN	RS
N S		K	P	E

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be in that position.

6. The device of claim 1 or 2 wherein the sequence comprises:

```

      10      20      30      40      50
    LYVDFRDVGWNDWIVAPPGYHAFYCHGECPPFLADHLNSTNHAIV
      K S S L QE VIS E FD Y E A AY MPESMKAS VI
      F E K I DN L N S Q ITK F P TL
      A S K
      60      70      80      90      100
    QTLVNSVNPCKIPKACCVPTELSAISMLYLDENENVVLKQDMVVEGCGCR
    SI HAI SEQV EP A EQMNSLAI FFNDQDK I RK EE T DA H H
    RF T S K DPV V Y N S H RN RS
    N S K P E

```

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be in that position.

7. The device of claim 1 or 2 wherein the sequence comprises:

```

      1      10      20      30      40
    Vg1 CKKRHLVVEFK-DVGWQNWVIAPQGYMANCYGECPPYPLTE
      50      60      70
    ILNGSN--H-AILQTLVHSIEPED-IPLPCCVPTKMSP
      80      90      100
    ISMLFYDNNNDNVVLRHYENMAVDECGCR

```

8. The device of claim 1 or 2 wherein the sequence comprises:

```

      1      10      20      30      40
    DPP CRRHSLYVDFS-DVGWDDWIVAPLGDAYYCHGKCPFLAD
      50      60      70
    HFNSTN--H-AVVQTLVNNNNPGK-VPKACCVPTQLDS
      80      90      100
    VAMLYLNDQSTVVLKQYQEMTVVGCGR

```

9. The device of claim 1 or 2 wherein the sequence comprises:

```

      1      10      20      30      40
    OP1 LYVSFR-DLGWQDWIIAPEGYAAYYCEGECAPPLNS
      50      60      70
    YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA
      80      90      100
    ISVLYFDDSSNVILKKYRNMVVRACGCH

```

10. The device of claim 1 or 2 wherein the sequence comprises:

-5
HQRQA

	1	10	20	30	40
OP1	CKKHELYVSFR-DLGWQDWIIAPEGYAAYYCEGECAPPLNS				
		50	60	70	
	YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA				
	80	90	100		
	ISVLYFDDSSNVILKKYRNMVVRACGCH				

11. The device of claim 1 or 2 wherein the sequence comprises:

	1	10	20	30	40
CBMP-2a	CKRHPLYVDFS-DVGWNDWIVAPPGYHAFYCHGECPPFLAD				
		50	60	70	
	HLNSTN--H-AIVQTLVNSVNS-K-IPKACCVPTELSA				
	80	90	100		
	ISMLYLDENEKVVVLKQDMVVEGCGCR				

12. The device of claim 1 or 2 wherein the sequence comprises:

	1	10	20	30	40
CBMP-2b	CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGDCPPFLAD				
		50	60	70	
	HLNSTN--H-AIVQTLVNSVNS-S-IPKACCVPTELSA				
	80	90	100		
	ISMLYLDEYDKVVVLKQEMVVEGCGCR				

13. The device of claim 1 or 2 wherein the sequence comprises:

	1	10	20	30	40
CBMP-3	CARRYLKVDFA-DIGWSEWIIISPKSFDAYYCSGACQFMPK				
		50	60	70	
	SLKPSN--H-ATIQSIVRAVGVPGIPEPCCVPEKMSS				
	80	90	100		
	LSILFFDENKNVVLKVYPNMTVESACR				

14. The device of claim 1 or 2 wherein the sequence comprises:

```
1      10      20      30      40
COP1  LYVDFQRDVGWDDWIIAPVDFDAYYCSGACQFPSAD
      50      60      70
      HFNSTN--H-AVVQTLVNNMNPBK-VPKPCCVPTLSA
      80      90      100
      ISMLYLDENSTVVLKKNYQEMTVVGCGCR
```

15. The device of claim 1 or 2 wherein the sequence comprises:

```
1      10      20      30      40
COP3  LYVDFQRDVGWDDWIVAPPGYQAFYCSGACQFPSAD
      50      60      70
      HFNSTN--H-AVVQTLVNNMNPBK-VPKPCCVPTLSA
      80      90      100
      ISMLYLDENEKVVLKKNYQEMVVEGCGCR
```

16. The device of claim 1 or 2 wherein the sequence comprises:

```
1      10      20      30      40
COP4  LYVDFS-DVGWDDWIVAPPGYQAFYCSGACQFPSAD
      50      60      70
      HFNSTN--H-AVVQTLVNNMNPBK-VPKPCCVPTLSA
      80      90      100
      ISMLYLDENEKVVLKKNYQEMVVEGCGCR
```

17. The device of claim 1 or 2 wherein the sequence comprises:

```
1      10      20      30      40
COP5  LYVDFS-DVGWDDWIVAPPGYQAFYCHGECFPPLAD
      50      60      70
      HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTLSA
      80      90      100
      ISMLYLDENEKVVLKKNYQEMVVEGCGCR
```

18. The device of claim 1 or 2 wherein the sequence comprises:

```
1      10      20      30      40
COP7  LYVDFS-DVGWDDWIVAPPGYHAFYCHGECFPPLAD
      50      60      70
      HLNSTN--H-AVVQTLVNSVNSKI--PKACCVPTLSA
      80      90      100
      ISMLYLDENEKVVLKKNYQEMVVEGCGCR
```

19. The device of claim 1 or 2 wherein the sequence comprises:

```

                                10
                                PKHHSQRARKKNKN
COP16   1      10      20      30      40
        CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGECPFPLAD
                                50      60      70
        HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA
                                80      90     100
        ISMLYLDENEKVVLKNYQEMVVEGCGCR

```

20. The device of claim 1 or 2 wherein the osteogenics protein comprises a pair of separate polypeptide chains.

21. Osteogenic protein, produced by expression of recombinant DNA in a host cell, capable of inducing endochondral bone formation in association with a matrix when implanted in a mammal.

22. A protein, produced by expression of recombinant DNA in a host cell, comprising one or more polypeptide chains less than about 200 amino acids long in a sequence sufficiently duplicative of the sequence of COP-5 or COP-7 such that said protein is capable of inducing cartilage formation in association with a matrix when implanted in a mammal.

23. The osteogenic protein of claim 21 having an apparent molecular weight of about 30 kD when oxidized as determined by comparison to molecular weight standards in SDS-polyacrylamide gel.

24. The osteogenic protein of claim 23 further characterized by being glycosylated.

25. The osteogenic protein of claim 21 having an apparent molecular weight of about 27 kD as determined by comparison to molecular weight standards in SDS-polyacrylamide gel electrophoresis.

26. The protein of claim 22 or 25 further characterized by being unglycosylated.

27. The protein of claim 21 or 22 comprising a pair of separate polypeptide chains.

28. The protein of claim 21 or 22 comprising the amino acid sequences:

```

      10      20      30      40      50
CXXXXLXVXFDXGWXXWXXXPXGXXAXYCXGXCPXXXXXXXNHAXX
      60      70      80      90     100
QXXVXXXNXXXPPXXCCXPXXXXXXXLXXXXXXVXLXXYXXMXVXXCXCX

```

wherein each X independently represents an amino acid.

29. The protein of claim 21 or 22 comprising the amino acid sequences:

```

      10      20      30      40      50
LXVXFDXGWXXWXXXPXGXXAXYCXGXCPXXXXXXXNHAXX
      60      70      80      90     100
QXXVXXXNXXXPPXXCCXPXXXXXXXLXXXXXXVXLXXYXXMXVXXCXCX

```

wherein each X independently represents an amino acid.

30. The protein of claim 21 or 22 comprising the amino acid sequences:

```

      10      20      30      40      50
CKRHPLYVDFRDVGWNDWIVAPPGYHAFYCHGECPPFLADHLNSTNHAIV
RRRS K S S L QE VIS E FD Y E A AY MPESMKAS VI
KE F E K I DN L N S Q ITK F P TL
Q A S K
      60      70      80      90      100
QTLVNSVNP GKIPKACCVPTELSAISMLYLDENENVVLKQDMVVEGCGCR
SI HAI SEQV EP A EQMNSLAI FFNDQDK I RK EE T DA H H
RF T S K DPV V Y N S H RN RS
N S K P E

```

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be in that position.

31. The protein of claim 21 or 22 comprising the amino acid sequences:

```

      10      20      30      40      50
LYVDFRDVGWNDWIVAPPGYHAFYCHGECPPFLADHLNSTNHAIV
K S S L QE VIS E FD Y E A AY MPESMKAS VI
F E K I DN L N S Q ITK F P TL
A S K
      60      70      80      90      100
QTLVNSVNP GKIPKACCVPTELSAISMLYLDENENVVLKQDMVVEGCGCR
SI HAI SEQV EP A EQMNSLAI FFNDQDK I RK EE T DA H H
RF T S K DPV V Y N S H RN RS
N S K P E

```

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be in that position.

32. The protein of claim 21 or 22 comprising the amino acid sequences:

```

      1      10      20      30      40
Vg1 CKKRHLVVEFK-DVGWQNWVIAPQGYMANCYGECPPYPLTE
      50      60      70
ILNGSN--H-AILQTLVHSIEPED-IPLPCCVPTKMSP
      80      90      100
ISMLFYDNNNDNVVLRHYENMAVDECGCR

```


33. The protein of claim 21 or 22 comprising the amino acid sequences:

	1	10	20	30	40
DPP	CRRHSLYVDFS-DVGWDDWIVAPLGYDAYYCHGKCPFLAD				
		50	60	70	
	HFNSTN--H-AVVQTLVNNNNPGK-VPKACCVPTQLDS				
	80	90	100		
	VAMLYLNDQSTVVLKQEMTVVGCGR				

34. The protein of claim 21 or 22 comprising the amino acid sequence:

	1	10	20	30	40
OP1	LYVSFR-DLGWQDWIIAPEGYAAYYCEGECAPFLNS				
		50	60	70	
	YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA				
	80	90	100		
	ISVLYFDDSSNVILKKYRNMVVRACGCH				

35. The protein of claim 21 or 22 comprising the amino acid sequences:

				-5	
				HQRQA	
	1	10	20	30	40
OP1	CKKHELYVSFR-DLGWQDWIIAPEGYAAYYCEGECAPFLNS				
		50	60	70	
	YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA				
	80	90	100		
	ISVLYFDDSSNVILKKYRNMVVRACGCH				

36. The protein of claim 21 or 22 comprising the amino acid sequences:

	1	10	20	30	40
CMP-2a	CKRHPLYVDFS-DVGWNDWIVAPPGYHAFYCHGECPPFLAD				
		50	60	70	
	HLNSTN--H-AIVQTLVNSVNS-K-IPKACCVPTLSA				
	80	90	100		
	ISMLYLDENEKVVVLKQDMVVEGCGCR				

37. The protein of claim 21 or 22 comprising the amino acid sequences:

```

1         10         20         30         40
CBMP-2b  CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGDCPFPLAD
              50         60         70
          HLNSTN--H-AIVQTLVNSVNS-S-IPKACCVPTELSA
              80         90        100
          ISMLYLDEYDKVVLKNYQEMVVEGCGCR

```

38. The protein of claim 21 or 22 comprising the amino acid sequences:

```

1         10         20         30         40
CBMP-3   CARRYLKVDFA-DIGWSEWIIISPKSFDAYYCSGACQFMPK
              50         60         70
          SLKPSN--H-ATIQSIVRAVGVPPIPEPCCVPEKMSS
              80         90        100
          LSILFFDENKNVVLKVYPNMTVESCACR

```

39. The protein of claim 21 or 22 comprising the amino acid sequences:

```

1         10         20         30         40
COP1     LYVDFQRDVGWDDWIIAPVDFDAYYCSGACQFPSAD
              50         60         70
          HFNSTN--H-AVVQTLVNNMNP GK-VPKPCCVPTELSA
              80         90        100
          ISMLYLDENSTVVLKNYQEMTVVGCGCR

```

40. The protein of claim 21 or 22 comprising the amino acid sequences:

```

1         10         20         30         40
COP3     LYVDFQRDVGWDDWIVAPPGYQAFYCSGACQFPSAD
              50         60         70
          HFNSTN--H-AVVQTLVNNMNP GK-VPKPCCVPTELSA
              80         90        100
          ISMLYLDENEKVVLKNYQEMVVEGCGCR

```

41. The protein of claim 21 or 22 comprising the amino acid sequences:

```

1         10         20         30         40
COP4     LYVDFS-DVGWDDWIVAPPGYQAFYCSGACQFPSAD
              50         60         70
          HFNSTN--H-AVVQTLVNNMNP GK-VPKPCCVPTELSA
              80         90        100
          ISMLYLDENEKVVLKNYQEMVVEGCGCR

```

42. The protein of claim 21 or 22 comprising the amino acid sequences:

```

1         10         20         30         40
COP5      LYVDFS-DVGWDDWIVAPPGYQAFYCHGECPFPLAD
              50         60         70
          HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA
              80         90        100
          ISMLYLDENEKVVVLKNYQEMVVEGCGCR

```

43. The protein of claim 21 or 22 comprising the amino acid sequences:

```

1         10         20         30         40
COP7      LYVDFS-DVGWNDWIVAPPGYHAFYCHGECPFPLAD
              50         60         70
          HLNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA
              80         90        100
          ISMLYLDENEKVVVLKNYQEMVVEGCGCR

```

44. The protein of claim 21 or 22 comprising the amino acid sequences:

```

              -10
              PKHHSSRARKKNKN
1         10         20         30         40
COP16     CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGECPFPLAD
              50         60         70
          HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA
              80         90        100
          ISMLYLDENEKVVVLKNYQEMVVEGCGCR

```

45. The protein of claim 21 or 22 comprising the product of expression of a DNA in a procaryotic cell.

46. A DNA sequence encoding an amino acid sequence sufficiently duplicative of that of the sequence encoded by the gene of Figure 1A such that said encoded sequence induces bone or cartilage formation when implanted in a mammal in association with a matrix.

47. The DNA of claim 46 encoding the same amino acid sequence as the gene set forth in Figure 1A.

48. The DNA sequence of claim 46 encoding:

```

      1      10      20      30      40
OPl  LYVSFR-DLGWQDWIIAPEGYAAYYCEGECAPPLNS
      50      60      70
      YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA
      80      90     100
      ISVLYFDDSSNVILKKYRNMVVRACGCH
```

49. The DNA sequence of claim 46 encoding:

```

                                     -5
                                     HQRQA
      1      10      20      30      40
OPl  CKKHELYVSFR-DLGWQDWIIAPEGYAAYYCEGECAPPLNS
      50      60      70
      YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA
      80      90     100
      ISVLYFDDSSNVILKKYRNMVVRACGCH
```

50. A cell line engineered to express the protein of claim 21 or 22.

51. The protein of claim 21 having a half maximum bone forming activity of about 20 - 25 ng per 25 mg of implant.

52. A biocompatible, in vivo biodegradable deglycosylated collagenous matrix defining pores of dimensions sufficient to permit influx, proliferation, and differentiation of migratory progenitor cells from the body of a mammal.

53. The matrix of claim 52 comprising close-packed particulate matter having a particle size within the range of 70-850 nm.

54. The matrix of claim 53 wher in said particulate matter has a particle size within the range of 70-420 mm.

55. The matrix of claim 52 defining a shape to span a non-union fracture in said mammal.

56. The matrix of claim 52 comprising demineralized, protein-extracted, deglycosylated, particulate xenogenic bone.

57. The matrix of claim 52 comprising a material selected from the group consisting of hydroxyapatite, tricalcium phosphate, polymers comprising lactic acid monomer units, polymers comprising glycolic acid monomer units, demineralized, guanidine-extracted, deglycosylated xenogenic bone, and mixtures thereof.

58. An osteogenic device for implantation in a mammal, said device comprising:

a biocompatible, in vivo biodegradable matrix defining pores of a dimension sufficient to permit influx, proliferation and differentiation of migratory progenitor cells from the body of said mammal; and

substantially pure osteogenic protein capable of inducing endochondral bone formation in said mammal disposed in said matrix and accessible to said cells.

59. The device of claim 1, 2, or 58 wherein said matrix comprises close-packed particulate matter having a particle size within the range of 70-850 mm.

60. The device of claim 1, 2, or 58 wherein said particulate matter has a particle size within the range of 70-420 mm.

61. The device of claim 1, 2, or 58 wherein said matrix comprises demineralized, protein-extracted, particulate, allogenic bone.

62. The device of claim 1, 2, or 58 wherein said matrix comprises a material selected from the group consisting of collagen, hydroxyapatite, tricalcium phosphate, polymers comprising lactic acid monomer units, polymers comprising glycolic acid monomer units, demineralized, guanidine-extracted allogenic bone, and mixtures thereof.

63. The device of claim 1, 2, or 58 wherein said matrix is shaped to span a non-union fracture in said mammal.

64. The device of claim 1, 2, or 58 disposed within the marrow cavity of allogenic bone.

65. The device of claim 1, 2, or 58 wherein said matrix comprises demineralized, protein extracted, particulate, deglycosylated xenogeneic bone.

66. The device of claim 65 wherein said matrix is treated with a protease.

67. The device of claim 58 wherein said osteogenic protein is unglycosylated.

68. The device of claim 67 wherein said osteogenic protein has an apparent molecular weight of about 27 kD when oxidized as determined by comparison to molecular weight standards in SDS-polyacrylamide gel electrophoresis.

69. The device of claim 58 wherein said osteogenic protein is glycosylated.

70. The device of claim 69 wherein said osteogenic protein has an apparent molecular weight of about 30 kD when oxidized as determined by comparison to molecular weight standards in SDS-polyacrylamide gel electrophoresis.

71. The device of claim 58 wherein said osteogenic protein comprises a pair of polypeptide chains.

72. The device of claim 71 wherein one chain of said pair of polypeptide chains has an apparent molecular weight of about 14 kD and the other has an apparent molecular weight of about 16 kD, both as determined after reduction by comparison to molecular weight standards in SDS-polyacrylamide gel electrophoresis.

73. The device of claim 71 wherein one chain of said pair of polypeptide chains has an apparent molecular weight of about 16 kD and the other has an apparent molecular weight of about 18 kD, both as determined after reduction by comparison to molecular weight standards in SDS-polyacrylamide gel electrophoresis.

74. The device of claim 58 wherein said osteogenic protein has the approximate amino acid composition set forth below:

<u>Amino acid</u> <u>residue</u>	<u>Rel. no.</u> <u>res./molec.</u>	<u>Amino acid</u> <u>residue</u>	<u>Rel. no.</u> <u>res./molec.</u>
Aspartic acid/	22	Tyrosine	11
Asparagine		Valine	14
Glutamic acid/	24	Methionine	3
Glutamine		Cysteine	16
Serine	24	Isoleucine	15
Glycine	29	Leucine	15
Histidine	5	Proline	14
Arginine	13	Phenylalanine	7
Threonine	11	Tryptophan	ND
Alanine	18		
Lysine	12		

75. The device of claim 58 wherein said osteogenic protein comprises the amino acid sequence:

VPKPCCAPT

76. The device of claim 1 or 58 wherein the half maximum bone inducing activity of said protein is 0.8 to 1.0 ng per mg of said matrix.

77. A method of inducing local cartilage or bone formation in a mammal comprising the step of implanting the device of claim 1, 2, or 58 in said mammal at a locus accessible to migratory progenitor cells of said mammal.

78. A method of inducing endochondral bone formation in a mammal comprising the step of implanting the device of claim 1 or 58 in said mammal at a locus accessible to migratory progenitor cells of said mammal.

79. A method of inducing endochondral bone formation in a non-union fracture in a mammal comprising the step of implanting in the fracture in said mammal the device of claim 63.

80. Antibodies reactive with an epitope of the protein of claim 21 or 22.